Effect of Phenformin on Water and Glucose Transport Across Isolated Human Ileum

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SUMMARY

The effect of phenformin on the uptake of glucose and water by isolated full-thickness sheets of human ileum has been studied. A significant decrease in mucosal glucose uptake was found, and significant glucose translocation to the serosal surface was abolished. This was accompanied by a proportional decrease in the production of lactate and in the net transport of water across the tissue. The magnitude of the observed changes does not support the hypothesis that overt glucose malabsorption is of significance in the therapeutic action of phenformin but confirms previous observations that the kinetics of absorption of a glucose load may be altered. DIABETES *22*:175-80, March, 1973.

It is commonly accepted that the antidiabetic action of phenformin differs qualitatively from the action of the sulfonylurea group of oral antidiabetic drugs.[1] It was originally suggested that it potentiated the action of insulin,[1] and interfered with tissue respiration by the inhibition of mitochondrial succinic oxidase.[2] While the hypothesis of interference with aerobic tissue respiration has received some support in the implication of the drug in episodes of lactic acidosis,[3] there is also some evidence for a site of action in the intestinal mucosa. Phenformin (phenethylbiguanide) flattened the response to oral glucose loads in normal subjects[4] and diabetic subjects,[5] while the response to intravenous glucose was unimpaired. Butylbiguanide was shown to diminish glucose uptake from in vivo perfused canine intestine,[5] and phenethylbiguanide inhibited glucose uptake in everted sacs of rat intestine.[6] Stowers and Bewsher[7] have produced some evidence of carbohydrate malabsorption in obese patients during phenformin therapy.

The development of a system for the measurement of net transport across isolated human intestine[8] offered the opportunity to test the effect of phenformin on human intestinal mucosa. The experimental system is adapted from the method of Fisher and Parsons,[9] in which physiological solutions are recirculated through the lumen of isolated intestine immersed in a physiological solution bathing the serosal surface. Fisher and Parsons[10] showed that, in isolated rat intestine, only a proportion of the glucose disappearing from the mucosal solution appeared in the serosal solution, and they were thus able to distinguish between glucose 'uptake' and 'translocation.' Fisher and Parsons also noted the dependence of water transport on the presence of glucose in the mucosal solution[11] in vitro, which has since been confirmed by other workers. More recently, a similar relationship between water absorption and luminal glucose concentration has been found in the perfused intact jejunum of human subjects,[12] but this relationship depends upon the concentration of luminal sodium. Thus the transport of glucose, water and sodium across the small intestine are mutually dependent processes, which are accompanied by an increased electro-potential difference across the tissue.[13] The system employed in the present experiments enables simultaneous measurement of these variables in isolated human tissue.

Ideally, such studies would be best performed on preparations of proximal small bowel mucosa. Our studies were carried out on ileum, since the required tissue was obtained at elective surgery and ileal surgery is much more frequent than jejunal surgery. Although most in vivo monosaccharide and water absorption takes place in the proximal small bowel,[14] in vitro studies have established that similar water and glucose transport can occur in the distal small bowel.[15] The tissue speci-

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mens were full-thickness, including the muscular layer; not only is removal of the musculature difficult, but there are good reasons for believing that rate-limiting factors with respect to transport across the isolated tissue are located in the mucosa rather than in the muscular layers.

METHODS

Segments (2.5 cm.) of fresh human ileum were obtained from surgical specimens removed during elective ileal surgical procedures on nineteen patients (table 1). All specimens were 'terminal ileum'—less than twelve inches from the ileocecal valve—except for two cases, in which the tissue was obtained from a site about two feet from the ileocecal valve. Histological inspection confirmed the absence of disease in the portion of tissue used for the experiment. The apparatus was mounted on a trolley fitted with a power input and gas cylinder, and each experiment was carried out within the operating theater suite. Tissue specimens were rapidly mounted in an acrylic tissue chamber and placed in an apparatus at 38° C., which permitted recirculation of physiological solution across the mucosal surface of the tissue, while the serosal surface was submerged in another physiological solution.[8] The mucosal solution was recirculated by a gas-lift using a mixture of 5 per cent CO_2 and 95 per cent O_2 (v/v). The tissue specimens were set up in warmed, oxygenated physiological media within seven minutes of interruption of the vascular supply.

TABLE 1

Clinical data

Clinical diagnosis		Operation
A.	*'Control' experiments (glucose in mucosal fluid only)*:	
A1	Ca. ascending colon and descending colon	Total colectomy
A2	Ca. cecum	R. hemicolectomy
A3	Ca. colon	R. hemicolectomy
B.	*Phenformin experiments (glucose in mucosal fluid only)*:	
B1	Ulcerative colitis	Proctocolectomy
B2	Crohn's dis. of colon	R. hemicolectomy
B3	Urinary incontinence	Cecocystoplasty
B4	Systolic urinary bladder	Cecocystoplasty
C.	*Glucose in mucosal and serosal fluids*:	
C1	Carcinoid tumor of small intestine	Ileal resection
C2	Ca. urinary bladder	Cecocystoplasty
C3	Ca. colon	R. hemicolectomy
*C4	Vesico-vaginal fistula	Ileal conduit
C5	Ulcerative colitis	Proctocolectomy
†C6	Interstitial cystitis	Cecocystoplasty
D.	*No glucose present*:	
D1	Ulcerative colitis	Proctocolectomy
D2	Ca. bladder	Ileal conduit
D3	Ca. cecum	R. hemicolectomy
D4	Ca. bladder	Cecocystoplasty
*D5	Crohn's dis.: term. ileum	Ileal resection
†D6	Interstitial cystitis	Cecocystoplasty

*Specimens obtained from mid-ileum.
†Tissue for these two experiments from the same patient.

The Krebs bicarbonate-Ringer solutions used in all experiments had the following ionic composition (in mEq./L.): Na^+ 146, K^+ 6, Mg^+ 1.2, Ca^+ 0.9, Cl^- 126, HCO_3^- 25, PO_4^- 1.2, SO_4^- 1.2. All mucosal solutions contained 500 mg. per cent (27.7 mM/L.) D-glucose initially; in some experiments serosal solutions contained the same concentration of glucose, otherwise they contained 27.7 mM/L. mannitol to preserve osmotic equilibrium between mucosal and serosal fluids. In four experiments phenformin hydrochloride (N'-β-phenethylbiguanide hydrochloride, 'Dibotin': Bayer Products Co.) was added to the mucosal solution at a concentration of 0.3 μg./ml., the latter concentration being equivalent to approximately 10 μg./gm. wet tissue (= 10 mg./kg.).

At the beginning of the experiment, both mucosal and serosal solutions were accurately delivered volumes. The mucosal fluid circulated for two hours, at the end of which the experiment was terminated, volume change estimated by a gravimetric method,[15] and samples taken for assay. Glucose and lactate concentrations in perfusion fluids and final fluid samples were measured in duplicate by standard enzymatic methods (Boehringer Mannheim). Tissue water changes were obtained gravimetrically, by wet and dry weight estimations of tissue before and after the experiments; in order to obtain dry tissue weight, specimens were incubated for forty-eight hours in an oven at 100° C. During the experiment, transmural potential difference was monitored through 2 per cent agar-Ringer bridges dipping into mucosal and serosal fluids and connected through standard calomel electrodes across the input terminals of a high impedance millivoltmeter (Vibron 33B electrometer, Electronic Instruments Ltd.). Mean transmural potential difference across the tissue in any single experiment was estimated from the mean of readings at fifteen-minute intervals.

In expressing the results, the convention was adopted of representing net change in either mucosal or serosal fluid during the experiment as a negative value for net decrease and a positive value for net increase of solute or solvent; thus the analogue of physiological absorption is negative change in the mucosal fluid and positive change in the serosal fluid, representing net transfer

across the tissue. Rate of change is expressed as microliters (water) or micromoles (solute) per square centimeter of mucosal surface per two hours (duration of experiment). Mean values have been calculated and compared using the non-paired Student t test.

RESULTS

Direct comparisons are made between 'control' experiments and 'phenformin' experiments; in both sets of experiments, the mucosal fluid was Krebs bicarbonate-Ringer solution containing 27.7 mM/L. D-glucose, and the serosal fluid was identical except for replacement of the glucose by 27.7 mM/L. mannitol. In 'phenformin' experiments, solutions were identical to 'control' experiments except that phenformin was added to the mucosal solution. Some results are cited from experiments in which mucosal and serosal fluids were identical, being either glucose-free or containing 27.7 mM/L. D-glucose.

Net Water Transport

The dependence of net water transport across in vitro intestine on the presence of glucose in the mucosal fluid is well established; the same phenomenon was confirmed in this preparation of isolated human ileum in a previous series of experiments,[16] and the results are shown in table 2a. The addition of equimolar glucose to both mucosal and serosal fluids increased net fluid loss from the mucosal fluid, and net fluid appearance in the serosal fluid; both these changes were significant ($P < 0.02$). Table 2a also shows that there was no significant change in glucose-dependent net water transport when the serosal glucose was replaced by equimolar mannitol. This is consistent with previous studies which have shown that net water transport is relatively unaffected by serosal glucose concentrations,[17] or even by the absence of serosal fluid.[18]

Table 2b shows the effect of the addition of phenformin to mucosal fluid containing glucose. There is a significant reduction in net water transport across the tissue.

Glucose Transport

There is a significant decrease in the uptake of glucose from the mucosal solution in the presence of phenformin, as shown in table 3a. There is a decrease in the appearance of glucose in the serosal fluid, representing glucose 'translocation,' which is not significant.

In table 3b, serosal glucose appearance in the presence of phenformin is compared with serosal glucose appearance in other experiments; these experiments include 'control' experiments and also experiments where there were variations in initial mucosal fluid tonicity. Variations in mucosal fluid tonicity alter net water transport across the tissue but do not change the net transport of glucose either in rat ileum,[15] or human ileum (Wingate, unpublished data). Comparison of serosal glucose appearance between 'control' experiments in table 3a and a larger series [group (i)] in table 3b shows little variation. A proportion of this glucose appearance is due to endogenous tissue glucose, as shown by experiments in which no glucose was added to either

TABLE 2

Net water transport

(a) Effect of glucose	N*	Mucosal fluid†	Serosal fluid†
no glucose in system	6	−47±18	+61±12
27.7 mM glucose in both fluids	6	−171±35	+162±32
27 mM glucose in mucosal fluid only	3	−189±22	+175±36
(b) Effect of phenformin	**N***	**Mucosal fluid†**	**Serosal fluid†**
(i) control‡	3	−189±22	+175±36
(ii) phenformin‡	4	−116±16	+79±8
(iii) significance (P) of difference between (i) and (ii)‡		<0.05	<0.05

*N = number of experiments.
†Net volume changes in mucosal and serosal fluids are mean values ± S.E.M. expressed as μL./cm.²/2 hrs.
‡In all experiments, the mucosal fluid contained 27 mM glucose, and the serosal fluid contained 27 mM mannitol.

TABLE 3

Net glucose transport

(a) Effect of phenformin			
Experiment	N*	Mucosal fluid†‡	Serosal fluid†‡
(i) control	3	−12.2±0.4	+1.9±0.5
(ii) phenformin	4	−9.6±0.3	+0.7±0.1
(iii) P for phenformin between (i) and (ii)		<0.01	0.1<P<0.05

(b) Serosal glucose appearance	N*	Serosal fluid† glucose	Significance of difference
27 mM/L. glucose in mucosal fluid initially	9	+1.7±0.2	P<0.01
no added glucose in mucosal or serosal fluid	7	+0.6±0.1	P>0.2
phenformin	4	+0.7±0.1	

*N=number of experiments.
†Net changes in mucosal and serosal fluids are mean values ±S.E.M. expressed in μM/cm.²/2 hrs.
‡Mucosal fluid with 27 mM./L. glucose, serosal fluid with 27 mM./L. mannitol.

mucosal or serosal fluid (group ii) in table 3b). Comparing the latter group with the phenformin experiments, it appears that the probable effect of phenformin was to abolish glucose 'translocation,' while reducing mucosal glucose uptake by about 25 per cent.

In these experiments, glucose transport was favored by an initial glucose concentration difference across the tissue of 27 mM/L.; this concentration difference does not affect the rate of mucosal glucose uptake since the rate of mucosal glucose uptake is 14.0 ± 0.7 μM/cm.^{2}/2 hr. when both mucosal and serosal fluid have an initial glucose concentration of 27 mM/L.

Lactate Production

Human ileum in vitro resembles rat intestine in vitro in the production of lactic acid, of which the greater part appears on the serosal side of the tissue.[15,19] Table 4 demonstrates this phenomenon in control experiments and shows that the formation of lactate is reduced by the addition of phenformin to the mucosal fluid. The reduction is significant in terms of the total production of lactate, and of the serosal lactate; the reduction in mucosal lactate appearance is not statistically significant. The ratio of serosal to mucosal lactate is virtually unchanged. It will be noted that the reduction in total lactate production due to phenformin is of a similar order of magnitude to the reduction in mucosal glucose uptake.

Transmural Potential Difference (P.D.)

The mean P.D. for control experiments was 10.6 ± 0.9 mv, while that for the phenformin series was 7.5 ± 1.2 mv; the reduction, although not statistically significant is of the same order of magnitude as the reductions in water transfer, glucose transfer and lactate production shown in tables 1-3.

TABLE 4
Lactate production

	(i) Control (N=3*)	(ii) Phenformin (N=4*)	(iii) Significance of difference between (i) and (ii)
Mucosal fluid†	5.1±2.7	3.6±0.5	—
Serosal fluid†	8.7±0.8	5.2±0.5	P<0.02
Total lactate‡	13.8±1.4	8.8±1.0	P<0.05
Lactate ratio§	1.7±0.1	1.5±0.3	—

*N=number of experiments.
†Mucosal fluid contained 27.7 mM/L. glucose, serosal fluid contained 27.7 mM/L. mannitol.
‡Lactate production rates expressed as μM/cm.^{2}/2 hr.
§Lactate ratio = $\frac{\text{serosal lactate rate}}{\text{mucosal lactate rate}}$

DISCUSSION

The presence of glucose in the intestinal lumen, or on the mucosal surface of the isolated preparations, is known to stimulate water absorption. Our observations demonstrate that phenformin, even at low concentrations, inhibits water absorption and glucose uptake across human intestinal mucosa. Kruger and co-workers[6] found glucose absorption was reduced by 38 per cent with a phenformin concentration of 10 mg./kg. The reduction found here is rather less, but the doses in the previous study, although similar, refer to pretreatment; in our experiments, the drug was added to the fluid bathing untreated normal mucosa.

The concomitant reduction in water transfer is consistent with the dependency of water transfer on luminal glucose shown by Sladen and Dawson;[12] their data suggest that K_m and V_{max} for human glucose absorption may be higher than for glucose absorption in the rat.[20]

It is of interest that phenformin appears to have depressed lactic acid formation in our preparation. It has been shown in isolated rat intestine that about 50 per cent of the absorbed glucose will appear as lactic acid.[19] Since one mole of glucose can produce two moles of lactic acid, it will be seen that we have found a similar relationship between glucose uptake and lactic acid production in our preparation. Although phenformin has been shown to inhibit aerobic tissue respiration in vitro[2] and has been implicated in clinical episodes of lactic acidosis,[3] we have found no evidence of this phenomenon in the intestinal mucosa. Without knowledge of the transport mechanisms in the intestinal mucosa, the mode of action of phenformin on the mucosa cannot be identified; it seems reasonable to speculate that the primary action of phenformin is on glucose uptake and that the effects on water transfer and lactate production are secondary to this. The effect on 'translocation' of glucose to the serosal surface may be no more than a reflection of reduced mucosal uptake, but the possibility of interference with the onward transport of glucose from the mucosa cannot be eliminated.

The significance of these findings in clinical terms must be interpreted with caution, even assuming that observations on the isolated intestine may be extrapolated to the intact normal or diabetic gut. The 'spare capacity' of the intestinal mucosa for both water and

glucose absorption is considerable; absorption of the glucose and water load of an average meal is usually accomplished in the proximal small bowel.[14] The likely intestinal effect of phenformin—if any—would be to delay the absorption of a glucose and water load both in terms of time and intestinal locus. This would produce the flattening effect on the absorption of a glucose load that has been reported.[4,5] The suggestion of glucose malabsorption derived from canine studies[5] seems unlikely in clinical practice, with therapeutic dosages of phenformin. Frank glucose malabsorption is extremely uncommon; a rare syndrome of glucose malabsorption has been reported and is manifested, as might be expected, by massive diarrhea.[21] It is of interest that phenformin overdosage induces gastrointestinal fluid loss unaccompanied by evidence of mucosal damage.[22]

Finally, it should be emphasized that even if phenformin induces altered kinetics of glucose and water absorption, this effect may be fortuitous and unrelated to the therapeutic action of the drug in diabetes mellitus.

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